

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Lam, et al.  
Serial No. : 09/914,543  
Filed : January 17, 2002  
Title : ENDOGLUCANASES

Art Unit : 1652  
Examiner : Manjunath N. Rao, Ph.D.

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Jay Short, am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as CEO and as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials.

2. I declare that the state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art for screening enzymes for endoglucanase and cellulase activity was very high. Procedures for making endoglucanase and cellulase enzyme fragments and sequence variations, e.g., with substitutions, deletions, insertions, and additions, were routine in the art at the time of the invention of the above-referenced patent application. Assays for identifying endoglucanase and cellulase enzyme fragments were conventional and routine in the art at the time of the invention. Assays for identifying variant polypeptides having endoglucanase and cellulase activity were conventional and routine in the art at the time of the invention. For example, assays for identifying polypeptides having endoglucanase activity is described in the specification, e.g., on page 17, line 6 to page 18, line 7. Dye-based techniques can be used in cup-plate diffusion assays with excellent sensitivity for the determination of endoglucanase activity in culture filtrates or during enzyme purification steps (see first paragraph, page 18), as further noted in Example 1, page 36. Endoglucanase activity assays also were well known in the art at the time of the invention, e.g., as described in USPNs 4,081,328; 4,904,599; 5,110,735; 5,366,884, to list only a few examples. Many of these assays could have been adapted and used in high-throughput screening assays, which were well known in the art at

the time of the invention. Accordingly, using the teaching of the specification one of ordinary skill in the art would have been able to routinely make and use the claimed genus of nucleic acids and polypeptides without undue experimentation.

3. I declare that use of high through-put screening assays is an example of the high state of art at the time of the invention for screening polypeptides for endoglucanase and cellulase enzyme activity. For example, at the time of the invention high through-put screening assays, including *in vitro* and *in vivo* (e.g., whole cell) nucleic acid expression and enzyme screening protocols were well known in the art. As noted above, the specification sets forth exemplary endoglucanase screening assays to determine if a polypeptide is within the scope of the claimed genus – and these or many of the other well known endoglucanase and cellulase assays could have been used in high through-put screening assays. Because screening assays known in the art at the time of the invention, including high through-put enzyme screening assays, predictably gave positive results (identifying enzymes with a desired activity) it would not have taken undue experimentation to make the genus of polypeptides of the invention.

4. Additionally, I declare that methods known at the time of the invention for modifying nucleic acid and polypeptide sequences in combination with high through-put enzyme screening known at the time of the invention, made methods that require previous knowledge of protein structure, including secondary or tertiary structure, active site sequences, and the like obsolete and unnecessary. It would not have been necessary for the skilled artisan to understand which regions of the enzyme could be modified to obtain a desired activity, e.g., to generate a variant a function or activity, or, which regions of the enzyme could be modified without loss of a function or activity. It would not have been necessary for the skilled artisan to understand which specific regions of enzyme sequence or structure needed to be modified without affecting function or activity to routinely generate the claimed genus of polypeptides. Methods for making and screening sequence modifications and enzyme fragments, including high through-put enzyme screening assays, were sufficiently comprehensive, routine and predictable at the time of the invention to predictably generate endoglucanase-encoding sequences without need of knowing which specific regions of a sequence or structure affected function or activity.

Accordingly, the specification provided sufficient guidance to one of ordinary skill in the art to make the genus of polypeptides to practice the invention.

5. I declare it was considered routine to design and screen for probes of varying lengths, for example, 15, 25, 35 or 50 nucleotides in length, that could hybridize under stringent conditions to a desired polynucleotide. Additionally, the specification, e.g., on page 8, line 17, to page 9, line 5, and page 20, lines 1 to 11, give guidance to the skilled artisan how to make nucleic acids that hybridize under stringent conditions to nucleic acids of the invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: \_\_\_\_\_

1/11/05

  
Jay Short

## CURRICULUM VITAE

**NAME** Jay M. Short, Ph.D.

Dr. Short is a founding member of Diversa Corporation, has served as Chief Technology Officer and Director of the company since its inception in 1994. He assumed the additional roles of President in 1998 and Chief Executive Officer in 1999. In February of 2000, Dr. Short led the company's highly successful initial public offering, which raised over \$200 million in gross proceeds – the largest biotechnology IPO ever completed at the time. Diversa was recently named one of the 100 most influential companies that will have the greatest influence on the future of human health. Diversa Corporation (NASDAQ: DVSA) is a leader in applying proprietary genomic technologies for the rapid discovery and optimization of novel products from genes and gene pathways.

### EDUCATION

2003	Certified Director Director Training Program The Anderson Graduate School of Management, University of California, Los Angeles
1981 - 1985	Ph.D., Biochemistry Case Western Reserve University, Cleveland, Ohio
1980 - 1981	Graduate Study, Macromolecular Science Case Western Reserve University, Cleveland, Ohio
1976 - 1980	B.A. with Honors, Chemistry Taylor University, Upland, Indiana

### RESEARCH & PROFESSIONAL EXPERIENCE

1999 - present	CEO and President Chief Technology Officer Board of Director Diversa Corporation San Diego, California
1998 - present	President and Chief Technology Officer Board of Director Diversa Corporation San Diego, California
1997 - 1998	Executive Vice President and Chief Technology Officer Board of Director Diversa Corporation San Diego, California
1994 - 1997	Chief Technology Officer Board of Director Diversa Corporation San Diego, California
1990 - 1994	President Stratacyte, Inc. La Jolla, California

Jay M. Short, Ph.D.

1992 - 1994	Vice President R&D (Research) and Operations Stratagene Cloning Systems La Jolla, California
1989 - 1992	Vice President R&D (Research) and Biological Operations Stratagene Cloning Systems La Jolla, California
1988 - 1989	Senior Staff Scientist Research and Development Stratagene Cloning Systems La Jolla, California
1985 - 1988	Staff Scientist Research and Development Stratagene Cloning Systems La Jolla, California
1981 - 1985	Ph.D. Research Case Western Reserve University Dr. Richard W. Hanson's Laboratory, Identification and characterization of the promoter for P-enolpyruvate carboxykinase. First identification of a cAMP regulatory domain. Cleveland, Ohio
1980 - 1981	Graduate Student Research Case Western Reserve University Dr. Bruce Roe's Laboratory, Analysis of the cellulase activity of <i>Trichoderma viride</i> . Cleveland, Ohio

## TEACHING EXPERIENCE

Thesis Advisor, University of Uppsala, Sweden, Ph.D. for Michelle Alting-Mees 1988-1993  
Lecturer, Committee for Advanced Scientific Education, Center for Drug Evaluation and Research, FDA 1992  
Faculty, Transgenic Mouse Model and Its Application in Assessing *In Vivo* Mutagenesis, Genetic Toxicology Workshop (3rd Annual) 1989  
Microbiological Associates Inc., Bethesda, MD.  
Faculty, DNA Cloning and Expression, Physiology Society Workshop, San Diego, CA. 1987  
Teaching Assistant, Molecular & Cellular Biology, Case Western Reserve University, Cleveland, OH. 1981-1985  
Teaching Assistant, Physiological Chemistry, Kent State University, Kent, OH. 1981  
Teaching Assistant, Quantitative Analysis, Taylor University, Upland, IN. 1978-1980

## CERTIFICATIONS

Certified Director	Director Training Program, University of California, Los Angeles, California The Anderson Graduate School of Management and The Harold Price Center
--------------------	--

Jay M. Short, Ph.D.

for Entrepreneurial Studies

PADI Diver Certification

## PROFESSIONAL EXPERIENCE

Diversa ranked # 2 among small companies for one of the best places for life scientists to work in this industry.  
Diversa named one of the 100 most influential companies that will have the greatest influence on the future of human health, Acumen 2004

Diversa's patent portfolio ranked # 1 on the 2003 Patent Scorecard by the MIT Survey

Largest Biotechnology IPO raising over \$200MM

Founding management member of Diversa Corporation

Board Director, Diversa Corporation, San Diego, CA

Board Director, Invitrogen Corporation, Carlsbad, CA

Board Director, Stressgen Biotechnologies, Vancouver, Canada and San Diego, CA

Board Director, Senomyx Corporation, San Diego, CA

Board Director, YPO (Young Presidents' Organization), San Diego, CA

Board Director & Treasurer, Stressgen Therapeutics, Victoria, BC, Canada

Board Director & Secretary, Stressgen Therapeutics, Victoria, BC, Canada

Board Director & Compensation Chairman, Victoria, BC, Canada

Board Member Advisor, Chemical and Engineering News

Board Member, BioCom San Diego

Board Advisor, IngleWood Ventures

Board of Advisors and Founding Member, Division of Biological Sciences, UCSD

Board Director and Executive Committee, Zymetrics

Fellow, Lifetime, The Explorers Club, New York, NY

Committee Member BioCom Science & Technology, San Diego

Consultant, Stratagene Cloning Systems, La Jolla, CA

Consultant, Micro Product Systems, Lynn, IN

Consultant for European Economic Community on Transgenic Toxicology Testing 1991-1994

Chairman, Discussion Group, Society of Toxicology Conference 1993

Editor, Mutation Research

Judge on the U.S. National Entrepreneur of the Year 2003

Institutional Animal Care and Use Committee (IACUC), Chairman and Institutional Official

NIEHS Peer Review Committee

Panel Member for Chemical Science & Technology for NIST, National Research Council 1997-2000

SBIR Study Section

Reviewer for U.S. Congressional Office of Technology Assessment (OTA) on The Human Genome Project and Patenting DNA Sequences.

Reviewer for Proceedings of the National Academy of Sciences, Genetic Analysis Techniques, Analytical Biochemistry & Nucleic Acids Research

U.S. Committee Member for Evaluation of Biotechnology Research in Spain

Visiting Scientist, International Centre of Insect Physiology and Ecology (ICIPE), Kenya 2002-2004

## MEMBERSHIPS

American Association for the Advancement of Science

American Chemical Society

American Men and Women of Science

American Society of Biochemistry and Molecular Biology

Jay M. Short, Ph.D.

American Society of Microbiology  
BioCom San Diego  
Environmental Mutagenesis Society  
Japanese Environmental Mutagen Society  
Science  
Society for Industrial Microbiology  
Society of Toxicology  
The Explorers Club, Fellow Lifetime Member, New York  
The New York Academy of Sciences  
YPO (Young Presidents' Organization) San Diego  
YPO (Young Presidents' Organization) International

## AWARDS

Henry F. Whalen, Jr. Award for Business Development, American Chemical Society, 2004  
Distinguished Alumnus Award for Professional Achievement, Taylor University, Upland, IN 2004  
Taylor University nomination for CCCU Award (Council for Christian Colleges & Universities) 2003  
Case Western Reserve University Alumni Profile 2003  
bioFusion 03 Breakthrough Innovation in Science Award Nomination 2003  
bioFusion 03 Life Science Leader of the Year Nomination 2003  
bioFusion 03 Life Science Company of the Year Nomination 2003  
ABL (Adaptive Business Leader) Innovations in HealthCare Gold Award 2003  
Deloitte and Touche's Orange County / San Diego Technology "Fast 50" 2003  
Finalists for UCSD Connect's Most Innovative New Product Award in the Biotechnology R&D Category 2002  
Deloitte and Touche "Fast 500" Technology 2002  
Deloitte and Touche's Orange County / San Diego Technology "Fast 50" 2002  
The Premier Print Award, Annual Report 2002  
Deloitte and Touche "Fast 500" Technology 2001  
Ernst & Young San Diego Entrepreneur of the Year 2001  
bioFusion 01 Life Science Innovator Award Nomination 2001  
T-Sector Life Science Innovator Award 2001  
Deloitte and Touche's Orange County / San Diego Technology "Fast 50" 2001  
San Diego Business Journal StarCom Honor 2001  
League of American Communication Professionals, Platinum Award, Annual Report 2001  
Ernst & Young Finalist for San Diego Entrepreneur of the Year in 2000  
The Premier Print Award, Annual Report 2001  
American Men and Women of Science 1995  
Who's Who Registry of Business Leaders 1994-1995  
SBIR Annual Report Program Success Profile (Top 8 of 800 Companies) 1993  
Stratagene Most Innovative Award - Managers/Supervisors 1992  
Stratagene Innovation Award - Big Blue® Transgenic Testing System 1991  
UCSD Connect Program 1<sup>st</sup> Place Award for Innovation and Entrepreneurship in Biotechnology 1991  
UCSD Connect Program 1<sup>st</sup> Place Award for Innovation and Entrepreneurship in Biotechnology 1990  
Stratagene Innovation Award - Lambda ZAP® vector 1990  
Stratagene Service Award 1990  
Award from the University of Victoria for Contributions to the Development of Short-term  
Transgenic Mutation Assays  
Nominated as Council Member for the U.S. Environmental Mutagen Society  
PNIT Patent Award

## MEDIA:

ABC Discovery News, ABC San Diego Channel 10, Agricultural Genomics, BBC Radio, Billings Gazette, BioCentury, Bioinformed Newsletter, BioPeople Magazine, BioTech Today Radio Show, Biotechnology Newsletter, BioVentures View, BioWorld Today, Business Daily, Business Week, CBS MarketWatch Weekend, CEO Cast, Chemical Engineering, Chemical Week, Chemistry & Industry (UK), Chemistry, CNBC, CNN Science & Technology, CNN Sunday Weekend, CNN WorldView, dBusiness.com, Digital Jam, Discovery Magazine, Drug Discovery Today, Elsevier Science Ltd., Forbes, Forbes.com, Fox CONNECT, Fox 6 News San Diego, German RTL TV, Good Morning America, Horizon Air Magazine, Idea TV, Inside Business Radio Show, JAG Financial News, KCRA Channel 3, KBPS Radio, KFMB Channel 8, KGTV Channel 10, KPBS, KUSI, Life Technology, London Financial Times, Los Angeles Times, Modern Drug Discovery, NBC San Diego Channel 7/39, National Geographic, National Radio Report, Nature, Nature Biotechnology, New York Times, PBS, Pirateinvestor.com, R&D Magazine, Reuters, San Diego Business Journal, San Diego Business Transcript, San Diego Magazine, San Diego Metropolitan, San Diego Union Tribune, SIM, Scientist, Specialty Chemicals, Sp2 Magazine, Stewards' Watch, T-Sector Magazine, The Age Magazine, The Economist, The Motley Fool, The Discovery Channel, The Discovery Channel, Time Magazine, USA Today, Wall Street Journal, Wall Street Transcript, Washington Post

## PATENTS

The Patent Scorecard for 2003 recognized Diversa's patent portfolio as being ranked # 1 by the MIT Survey. This ranking provides an overall assessment of a company's intellectual property power. This measure showcases the broader significance of a company's patents by examining how often its U.S. patents from the previous five years are cited as prior art in the current year's batch. A value of 1.0 represents average citation frequency, so, for example, a value of 1.4 would indicate a company's patents were cited 40 percent more often than the average. Diversa has a value of 14.43.

DNA Cloning Vectors with *in vivo* Excisable Plasmids 1987  
Mutagenesis Testing Using Transgenic Animals Carrying Marker Genes 1987  
Mutagenesis Testing Using Transgenic Non-Human Animals Carrying Test DNA Sequences 1987  
Dietary and Hormonal Regulation of Expression of Exogenous Genes in Transgenic Animals Under Control of the Promoter of the Gene  
Phosphoenolpyruvate Carboxykinase 1988  
A Transgenic Mouse for Measurement and Characterization of Mutation Induction *In Vivo* 1989  
Rapid Screening Mutagenesis and Teratogenesis Assay 1989  
A Combinatorial Approach to Regenerating the Immunoglobulin Repertoire in Prokaryotic Cells 1990  
Transgenic Animal Models for *In Vivo* Mutagenesis Testing 1990  
Polycos Vectors 1991  
A Lambda Packaging Extract Lacking  $\beta$ -Galactosidase Activity 1991  
A System for Regulation of Eukaryotic Genes 1991  
Methods for Phenotype Creation from Multiple Gene Populations 1991  
Transgenic Non-Human Animals Carrying Test DNA Sequences 1992  
Mutagenesis Testing Using Transgenic Non-Human Animals Carrying Test DNA Sequences 1992  
Selectable System Patent 1992  
Polycos Mutagenesis Systems 1993  
Use of Trans-acting Proteins for the Development of an *In Situ* Expression Screening System 1993  
Enzyme Kits and Libraries 1995  
Enzyme Activity Screening of Clones having DNA from Uncultivated Microorganisms 1995  
Enzyme Tiered 1995  
Method for Screening for Enzyme Activity 1995  
Combined Enzyme Screening/Evolution 1995  
Uncultured/Activity Screening 1995  
Directed Evolution of Thermophilic Proteins 1995  
Combinatorial Enzyme Development (Directed Mutagenesis) 1996  
Protein Activity Screening of Clones having DNA from Uncultivated Microorganisms 1996  
Production and Use of Normalized DNA Libraries 1996



Methods of DNA Shuffling with Polynucleotides Produced by Blocking or Interrupting a Synthesis or Amplification Process 1996  
Method of Screening for Enzyme Activity (Biopanning) 1996  
Directed Evolution of Thermophilic Enzymes 1996  
Environmental Biopanning 1996  
Combinatorial Enzyme Development 1996  
Enzyme Activity Screening of Clones Having DNA from Uncultivated Microorganisms 1996  
Normalized Samples/Libraries 1996  
Reassembled Pools of Mutagenized DNA & Procedure 1996  
Fluorescent-based Single Screening for Enzymes 1996  
High Throughput Screening for Novel Enzymes 1997  
Nucleotide Sequence of the *Aquifex aeolicus* Genome, Fragments Thereof, and Uses Thereof 1997  
Screening for Novel Bioactivities 1997  
Screening for Novel Compounds which Regulate Biological Interactions 1997  
Method for Screening Enzyme Activity 1997  
High Throughput Screening for Novel Enzymes 1997  
"Discovery" (whole process, including uncultivated, normalized, biopanning, screening, evolving, (etc.) 1997  
Production of Enzymes Having Desired Activities By Mutagenesis 1999  
Protein Activity Screening of Clones Having DNA from Uncultivated Microorganisms 1999  
Method of DNA Reassembly by Interrupting Synthesis 1999  
Production and Use of Normalized DNA Libraries 1999  
Enzyme Kits and Libraries 1999  
Screening for Novel Bioactivities 2000  
Method for Screening for Enzyme Activity 2000  
Screening for Novel Bioactivities 2000  
Production and Use of Normalized DNA Libraries 2000  
Method of Screening for Enzyme Activity 2000  
Screening Methods for Enzymes and Enzyme Kits 2001  
Saturation Mutagenesis in Directed Evolution 2001  
High Throughput Screening for Novel Enzymes 2001  
Recombinant Bacterial Phytases and Uses Thereof 2001  
Methods Useful for Nucleic Acid Sequencing Using Modified Nucleotides Comprising Phenylboronic Acid 2001  
End Selection in Directed Evolution 2001  
Gene Expression Library Produced From DNA From Uncultivated Microorganisms and Method for Making the Same 2001  
Directed Evolution of Thermophilic Enzymes 2002  
Method for Screening for Enzyme Activity 2002  
Exonuclease-Mediated Gene Assembly in Directed Evolution 2002  
End Selection In Directed Evolution 2002  
Exonuclease-Mediated Gene Assembly in Directed Evolution 2002  
Screening for Novel Bioactivities 2002  
Method of DNA Shuffling with Polynucleotides Produced or Blocking or Interrupting Synthesis or Amplification Process 2002  
Production and Use of Normalized DNA Libraries 2002  
Sequence Based Screening 2002  
Non-Stochastic Generation of Genetic Vaccines 2002  
Altered Thermostability of Enzymes 2003  
Screening Methods for Enzymes and Enzyme Kits 2003  
Methods for Identifying a Desired Enzymatic Activity 2003  
Enzymes Kits and Libraries 2003  
Method for Screening for Enzyme Activity 2003  
Protein Activity Screening of Clones having DNA from Uncultivated Microorganisms 2003  
High Throughput Screening of Mycelia for Bioactivities of Biomolecules 2003  
Screening for Novel Bioactivities 2003  
Coated Surfaces for Selective Enrichment of Microbial Populations 2003

Recombinant Bacterial Phytases and Uses Thereof 2003  
Synthetic Ligation Reassembly in Directed Evolution 2003  
Process for Generating Optimized Molecules from a Manmade Library of Polynucleotides made by Combinatorial Saturation Mutagenesis (amended) 2003  
Exonuclease-Mediated Nucleic Acid and Reassembly in Directed Evolution 2003  
Methods for Purifying Annealed Doubled-Stranded Oligonucleotides Lacking Base Pair Mismatches 2004  
End Selection in Directed Evolution 2004  
Protein Activity Screening of Clones having DNA from Uncultivated Microorganisms 2004  
Method of Screening for Enzyme Activity 2004  
Exonuclease-Mediated Gene Assembly in Directed Evolution (3/23/04 new issuance) 2004  
Directed Evolution of Thermophilic Enzymes (3/30/04 new issuance) 2004  
Non-Stochastic Generation of Genetic Vaccines and Enzymes 2004  
Directed Evolution of Thermophilic Enzymes 2004  
Over 350 Additional Pending Patent Applications Worldwide.

## GRANTS AND CONTRACTS

\*Phase I Small Business Contract #N43-Am-62282. 1985-1986 P.I.  
Vectors and Techniques for Rapid DNA Sequencing  
\*Phase II Small Business Contract #N43-Am-62282. 1988-1990 P.I.  
Vectors and Techniques for Rapid DNA Sequencing  
\*Phase I Small Business Grant 2R43ES04484-02. 1986-1987 P.I.  
Identification of Genetic Lesions Leading to Mutations  
\*Phase II Small Business Grant 2R43ES04484-02. 1989-1992 P.I.  
Identification of Genetic Lesions Leading to Mutations  
\*1R01-ES04728-01A1. 1989-1992. (NIEHS) P.I.  
Animal Model for Identification of Genetic Lesions  
\*Phase I Small Business Grant #R43GM42291-01. 1989 P.I.  
Switch Mechanism for Gene Expression in Transgenics  
\*RFP NIH-ES-88-11. 1989-1994. (NIEHS) Co-I.  
Development of Mutagenesis Assays Using Transgenic Mice  
\*Phase II Small Business Grant #2R44GM42291-02. 1990-1992 (DRG/NIH) P.I.  
Switch Mechanism for Gene Expression in Transgenics  
\*Phase I Small Business Grant #1R43GM46585-01. 1991 (DRG/NIH) P.I.  
Generation of a Peptide Screening System Through the Development of  
Combinatorial-splicing "Polycos" Vectors  
\*Phase I Small Business Grant #1R43CA57066-01. 1992 (NCI) P.I.  
Transgenic Rats: A Short-term Mutagenicity Assay for Multi-species Testing of Suspected Human Carcinogens  
\*Phase I Small Business Grant #1R43GM48300-01. 1992. (DRG/NIH) P.I.  
Analysis of the Immunoglobulin Hypermutator Mechanism  
\*Phase I Small Business Grant #1R43ES06146-01. 1992 (NIEHS) P.I.  
Selectable "Polycos" Shuttle Vectors for In Vivo Mutagenicity Testing  
\*Phase II Small Business Grant #2R44GM46585-02. 1992-1994 (NIGMS) P.I.  
Peptide Screening Utilizing Combinatorial Polycos Vector  
\*Phase I Small Business Grant #1R43RR08667-01. 1992-1993 (DRG/NIH) Co-I.  
A One-step PCR Cloning System Based on FLP Recombination  
\*Phase II Small Business Grant #2R44CA57066-02. 1993-1995 (NCI) P.I.  
Transgenic Rats: Transgenic Rat Model for Mutagenicity Testing  
\*Phase I Small Business Grant. 1993-1994 (NIH) Co-I.  
Transgenic Fish Model for Mutagenicity Testing  
\*Phase II Small Business Grant 1994-1996 (NIH) P.I.

Jay M. Short, Ph.D.

"Polycos" Shuttle Vectors for Mutagenicity testing  
\*Phase I Small Business Grant. 1994 (NIH) Co-I.  
Vector System for Studying Protein-Protein Interactions  
\*CRADA with LLNL. 1994 (NIH) Co-I.  
Mouse and Rat Painting Probes  
\*CRADA with FDA. 1994 (NIH) Co-I.  
Tamoxifen Testing in F-344 Rats  
\*CRADA with NASA. 1994 (NIH) Co-I.  
Radiation Damage in the Microgravity Environment

## ABSTRACTS AND INVITED LECTURES:

Over 200 Abstracts and Invited Lectures.

## PUBLICATIONS:

1. Yoo-Warren, H., Monahan, J.E., Short, J.M., Short, H., Bruzel, A., Wynshaw-Boris, A., Meisner, H.M., Samols, D., and Hanson, R.W. (1983) Isolation and Characterization of the Gene Coding for Cytosolic Phosphoenolpyruvate Carboxykinase (GTP) from the Rat. *Proc. Natl. Acad. Sci. U.S.A.*, 80:3656-3660.
2. Wynshaw-Boris, A., Lugo, T.G., Short, J.M., Fournier, R.E.K., and Hanson, R.W. (1984) Identification of cAMP Regulatory Region in the Gene for Rat Cytosolic Phosphoenolpyruvate Carboxykinase (GTP): Use of Chimeric Genes Transfected into Hepatoma Cells. *J. Biol. Chem.*, 259:12161-12169.
3. Wynshaw-Boris, A., Lugo, T.G., Short, J.M., Fournier, R.E.K., and Hanson, R.W. (1985) A Region of the Gene for Rat Cytosolic P-enolpyruvate Carboxykinase Confers cAMP Responsiveness to the HSV-thymidine Kinase Gene. In: *Membrane Receptors and Cellular Recognition*, (M. Czech and C.R., Kahn, eds.), Alan Liss Inc., New York, pp 339-346.
4. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1986) Characterization of the Phosphoenolpyruvate Carboxykinase (GTP) Promoter-Regulatory Region. I. Multiple Hormone Regulatory Elements and the Effects of Enhancers. *J. Biol. Chem.*, 261:9714-9720.
5. Short, J.M., Wynshaw-Boris, A., Short, H.P., and Hanson, R. W. (1986) Characterization of the Phosphoenolpyruvate Carboxykinase (GTP) Promoter-Regulatory Region. II. Identification of cAMP and Glucocorticoid Regulatory Domains. *J. Biol. Chem.*, 261:9721-9726.
6. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1986) The Determination of Sequence Requirements for Hormonal Regulation of Gene Expression. *Biotechniques*, 4:104-119.
7. Burns, D.M., Bhandari, G., Short, J.M., Sanders, P.G., Wilson, R.H., and Miller, R.E. (1986) Selection of a Rat Glutamine Synthetase cDNA Clone. *Biochemical and Biophysical Research Communications*, 134:146-151.
8. Hod., Y. Cook, J.S., Weldon, S.L., Short, J.M., Wynshaw-Boris, A., and Hanson, R.W. (1986) Differential Expression of the Genes for the Mitochondrial and Cytosolic Forms of P-enolpyruvate Carboxykinase Gene. In: *Metabolic Regulation: Application of Recombinant DNA Techniques*, (A.G., Goodridge and R.W. Hanson eds.), Annals of the New York Academy of Sciences, New York, Vol. 278, pp. 31-45.
9. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1987) *cis* - acting Regulatory Elements in Hormonally Responsive Genes. In: *Progress in Nucleic Acid Research and Molecular Biology* (W.E. Cohn and K. Moldave eds.), Academic Press, Inc., Orlando, Florida, 34:59-87.

10. Bullock, W., Fernandez, J.M., and Short, J.M. (1987) XL1-Blue: A High Efficiency Plasmid Transforming *recA E.coli* Strain With  $\beta$ -Galactosidase Selection. *Biotechniques*, 5:60-64.
11. Short, J.M., Fernandez, J.F., Sorge, J.A., and Huse, W. (1988) Lambda ZAP<sup>®</sup>: A Bacteriophage Lambda Expression Vector With *In Vivo* Excision Properties. *Nucleic Acids Res.*, 16:7583-7600.
12. Short, J.M. (1988) Book Review: Vectors - A Survey of Molecular Cloning Vectors and Their Uses. Raymond L. Rodriques and David T. Denhardt, eds, Butterworths, Stoneham, MA. *Genomics*, 2:270-271.
13. Short, J.M., and Pollard, A. (1988) Gigapack XL: Size Selective Packaging Extract. *Strategies in Mol. Biol.*, 1:5-7.
14. Kretz, P.L., and Short, J.M. (1989) Gigapack II: A Restriction Deficient (*mcrA*-, *B*-, *hsd*-, *mrr*-) Lambda Packaging Extract. *Strategies in Mol. Biol.*, 2(2):25-26.
15. Kretz, P.L., Reid, C.H., Greener, A., and Short, J.M. (1989) Effect of Lambda Packaging Extract M<sub>cr</sub> Restriction Activity on DNA Cloning. *Nucleic Acids Res.* 17:5409.
16. Sastry, L., Alting-Mees, M., Huse, W.D., Short, J.M., Sorge, J.A., Hay, B.N., Janda, K.D., Benkovic, S.J., and Lerner, R.A. (1989) Cloning of the Immunological Repertoire in *E. coli* for Generation of Monoclonal Catalytic Antibodies I. Construction of a V<sub>H</sub> Specific cDNA Library. *Proc. Natl. Acad. Sci. U.S.A.*, 86:5728-5732.
17. Short, J.M. (1989) The Use of Lambda Phage Shuttle Vectors in Transgenic Mice for Development of a Short Term Mutagenicity Assay. In: *Fifth International Conference on Environmental Mutagens*, Alan Liss, Inc., New York, Part A:335-367. Article and Lecture.
18. Alting-Mees, M., and Short, J.M. (1989) pBluescript II: Gene Mapping Vectors. *Nucleic Acids Res.*, 17:9494.
19. Shopes, B., Alting-Mees, M., Amber, J.R., Ardourel, D., Callahan, M., Detrick, J., Hay, B.N., Hogrefe, H.H., Greener, A., Gross, E.A., Kubitz, M.M., Mullinax, R.L., Wilson, C., Short, J.M., and Sorge, J.A. (1990) Bacteriophage Immuno-expression Libraries: An Emerging Technology for the Identification and Production of Monoclonal Antibodies. *Antibody Engineering, New Tech. & Application Implications*. pp. 98-101.
20. Alting-Mees, M., Amberg, J., Ardourel, D., Elgin, E., Greener, A., Gross, E.A., Kubitz, M., Mullinax, R.L., Short, J.M., and Sorge, J.A. (1990) Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas. *Strategies in Mol. Biol.*, 3:1-9.
21. Kohler, S., Provost, S., Dyaico, M., Sorge, J., and Short, J.M. (1990) Development of a Short-term, *In Vivo* Mutagenesis Assay: The Effects of Methylation on the Recovery of a Lambda Phage Shuttle Vector from Transgenic Mice. *Nucleic Acids Res.*, 18:3007-3013.
22. Kohler, S., Provost, G.S., Kretz, P.L., Fieck, A., and Short, J.M. (1990) An *In Vivo* Assay Using Transgenic Mice to Analyze Spontaneous and Induced Mutations at the Nucleic Acid Level. *Strategies in Mol. Biol.*, 3:19-21.
23. Kretz, P., Kohler, S., and Short, J.M. (1990) The Effect of *E. coli* Minute 98 Locus on DNA Containing Eukaryotic Modifications. *Strategies in Mol. Biol.*, 3:21-22.
24. Mullinax, R.L., Gross, E.A., Amberg, J., Hogrefe, H., Kubitz, M., Greener, A., Alting-Mees, M., Ardourel, D., Hay, B.N., Short, J.M., Sorge, J.A., and Shopes, B. (1990) Identification of Human Antibody Fragment Clones Specific for Tetanus Toxin in a Bacteriophage Lambda Immuno-Expression Library. *Proc. Natl. Acad. Sci. U.S.A.*, 87:8095-8099.

25. Cline, J., Lundberg, K., Nielson, K., Sorge, A., Short, J.M., and Mathur, E.J. (1990) StrataClean Resin: Non-Toxic Protein Extraction. *Strategies in Mol. Biol.*, 4(4):49-51.
26. Mullinax, R.L., Gross, E.A., Amber, J.R., Hay, B.N., Hogrefe, H.H., Kubitz, M.M., Greener, A., Alting-Mees, M., Ardourel, D., Short, J.M., Sorge, J.A., and Shopes, B. (1990) Human Antibody Clones Isolated From a Bacteriophage Lambda Immunoexpression Library. *Strategies in Mol. Biol.*, 4(4):51-52.
27. Provost, G.S., Kohler, S.W., Fieck, A., Kretz, P.L., Molina, T., and Short, J.M. (1990) Short-term Germ Line and Somatic Cell Mutagenesis Testing With *LacI* Lambda Phage Shuttle Vectors in Transgenic Mice. *Strategies in Mol. Biol.*, 4(4):55-56.
28. Kohler, S.W., Provost, G.S., Kretz, P.L., Fieck, A., Sorge, J.A., and Short, J.M. (1990) The Use of Transgenics Mice for Short Term, *In Vivo* Mutagenicity Testing. *Genetic Analysis Techniques*, 7(8):212-218.
29. Shopes, B., Mullinax, R.L., Amber, J.R., Gross, E.A., Hay, B.N., Hogrefe, H.H., Kubitz, M.M., Greener, A., Alting-Mees, M., Ardourel, D., Short, J.M., and Sorge, J.A. (1990) ImmunoZAP<sup>®</sup> Bacteriophage Libraries: A New Technology for the Identification and Expression of Monoclonal Antibodies. *Biotech USA Conference Proceedings*, pp.332-341.
30. Raleigh, E.A., Benner, J., Bloom, F., Braymer, H.D., DeCruz, E., Dharmalingam, K., Heitman, J., Noyer-Weidner, M., Piekarowicz, A., Kretz, P.L., Short, J.M., and Woodcock, D. (1991) Nomenclature Relating to Restriction of Modified DNA in *Escherichia coli*. *Journal of Bacteriology*, 173(8):2707-2709.
31. Kretz, P., Kohler, S., and Short, J.M. (1991) Identification and Characterization of a Gene Responsible for Inhibiting Propagation of Methylated DNA Sequences in *mcrA*, *mcrB1* *E. coli* Strains. *Journal of Bacteriology*, 173:4707-4716.
32. Kohler, S.W., Provost, G.S., Fieck, A., Kretz, P.L., Bullock, B., Sorge, J. A., Putman, D., and Short, J.M. (1991) Spectra of Spontaneous and Induced Mutations Using a Lambda ZAP<sup>®</sup> *LacI* Shuttle Vector in Transgenic Mice. *Proc. Natl. Acad. Sci. U.S.A.*, 88(18):7958-7962.
33. Wyborski, D., and Short, J.M. (1991) Analysis of Inducers of the *E.coli* *Lac* Repressor System in Mammalian Cells and Whole Animals. *Nucleic Acids Research*, 19:4647-4653.
34. Lundberg, K.L., Shoemaker, D.D., Adams, M.W.W., Short, J.M., Sorge, J.A., and Mathur, E.J. (1991) High Fidelity Amplification With a Thermostable DNA Polymerase Isolated from *Pyrococcus Furiosus*. *Gene*, 108:1-6.
35. Kohler, S.W., Provost, G.S., Fieck, A., Kretz, P.L., Bullock, W.O., Putman, D.L., Sorge, J.A., and Short, J.M. (1992) Analysis of Spontaneous and Induced Mutations in Transgenic Mice Using a Lambda ZAP<sup>®</sup>/*LacI* Shuttle Vector. *Environmental and Molecular Mutagenesis*, 18:316-321.
36. Fieck, A., Wyborski, D., and Short, J.M. (1992) Modifications of the *E. coli* *Lac* Repressor for Expression in Eukaryotic Cells: Effects of Nuclear Signal Sequences on Protein Activity and Nuclear Accumulation. *Nucleic Acids Research*, 20:1785-1791.
37. Hay, B., and Short, J.M. (1992) ExAssist<sup>TM</sup> Helper Phage and SOLR<sup>TM</sup> Cells for Lambda ZAP<sup>®</sup> II Excisions. *Strategies in Mol. Biol.*, 5:16-18.
38. Short, J.M. (1992) Tissue Specific Mutagenesis in Transgenic Mice. *The Toxicology Forum, 1992 Annual Winter Meeting*, pp.79-109.
39. Alting-Mees, M.A., Sorge, J.A., and Short, J.M. (1992) pBluescript II: Multifunctional Cloning and Mapping Vectors. *Methods in Enzymology*, 216:483-495.

40. Short, J.M., and Sorge, J.A. (1992) *In Vivo* Excision Properties of Bacteriophage Lambda ZAP® Expression Vectors. *Methods in Enzymology*, 216:495-508.
41. Short, J.M. (1992) Transgenic Animals for Carcinogenicity and Genotoxicity Testing. *Biotechnology International, The Global Review of Industry Manufacture and Application 1992*. Section.2. pp. 91-99.
42. Provost, G.S., Hamner, R., Kretz, P.L., and Short, J.M. (1992) Response to the Commentary Article: Comparison of Mutation Frequencies Obtained Using Transgenes and Specific-locus Mutation Systems in Male Mouse Germ Cells. *Mutation Research*, 298:145-147.
43. DuCoeur, L.C., Wyborski, D.L., and Short, J.M. (1992) Control of Gene Expression in Eukaryotic Cells Using the Lac Repressor System. *Strategies in Mol. Biol.*, 5(3):70-72.
44. Jerpseth, B., Greener, A., Short, J.M., Viola, J., and Kretz, P.L. (1992) XL1-Blue MRF: McrA-, McrCB-, Mrr-, HsdRMS- derivative of XL1-Blue. *Strategies in Mol. Biol.*, 5(3):81-83.
45. Alting-Mees, M., Hoener, P., Ardourel, D., Sorge, J., and Short, J.M. (1992) ZAP Express™ and pBK-CMV, pBK-RSV Phagemid Vectors for Prokaryotic and Eukaryotic Expression. *Strategies in Mol. Biol.*, 5(3):58-61.
46. Short, J.M., Provost, G.S., Kretz, P.L., and Dyaico, M.J. (1992) Overview of the Big Blue® *In Vivo* Mutagenesis Assay. *Mammalian Mutagenesis Study Group Communication – JEMS.MMS*, 6:73-89.
47. Lundberg, K.S., Kretz, P.L., Provost, G.S., and Short, J.M. (1993) The Use of Selection in Recovery of Transgenic Targets for Mutation Analysis. *Mutation Research Letters*, 301/2:99-105.
48. Mirsalis, J., Provost, G.S., Matthews, C., Hamner, R., Schindler, J.E., O'Loughlin, K., MacGregor, J.T., and Short, J.M. (1993) Induction of Hepatic Mutations in *LacI* Transgenic Mice. *Mutagenesis*, 8:265-271.
49. Alting-Mees, M.A., Vaillancourt, P., and Short, J.M. (1993) Phagemids and Other Hybrid Vectors. In: *Plasmids: A Practical Approach*. (ed. K. Hardy). IRL Press, pp. 197-223.
50. Provost, G.S., Kretz, P.L., Dyaico, M., Lundberg, K., and Short, J.M. (1993) Transgenic Systems for *In Vivo* Mutation Analysis. *Mutation Research*, 288:133-149.
51. Hedden, V., Callen W., Short, J.M., and Kretz, K. (1993) Improved Sequence Analysis of Mutations Identified With the Big Blue® System. *Strategies in Mol. Biol.*, 6:27-28.
52. Jerpseth, B., Greener, A., Short, J.M., Viola, J., and Kretz, P.L. (1993) New Restriction-Minus Derivatives of XL1-Blue *E. coli* Cells. *Strategies in Mol. Biol.*, 6:24.
53. Vaillancourt, P., Wyborski, D.L., and Short, J.M. (1993) The FLASH® CAT Kit: A Fast, Sensitive CAT Assay Without Radioactivity. *Strategies in Mol. Biol.*, 5:17-19.
54. Short, J.M., Dyaico, M.J., Provost, G.S., Kretz, P.L., Rogers, B.J., Ardourel, D., Wyborski, D.L. and Moores, J.C. (1993) Transgenic Mice and Rats for Tissue Specific Mutation Analysis. *JEMS*, 22:45-46.
55. Alting-Mees, M.A., and Short, J.M. (1993) Polycos Vectors: Filamentous Phage Packaging Using Lambda Extracts. *Gene*, 137:93-100.
56. Piegorsch, W.W., Lockhart, A.C., Margolin, B.H., Tindall, K.R., Gorelick, N.J., Short, J.M., Carr, G.J., and Shelby, M.D. (1994) Sources of Variability in Data from a *lacI* Transgenic Mouse Mutation Assay. *Environmental & Molecular Mutagenesis*, 23:17-31.

57. Dyaico, M., Provost, G.S., Kretz, P.L., Ransom, S.L., Moores, J.C. and Short, J.M. (1994) The Use of Shuttle Vectors for Mutation Analysis in Transgenic Mice and Rats. *Mutation Research*, 307:461-478.
58. Wyborski, D.L., Malkhosyan, S., Moores, J.C., Dyaico, M.J., and Short, J.M. (1994) Rat2 Cell Line for *In Vitro* Mutagenicity Testing. *Strategies in Mol. Biol.*, 7(2):55-56.
59. Provost, G.S. and Short, J.M. (1994) Characterization of Mutations Induced by Ethylnitrosourea in Seminiferous Tubule Germ Cells of Transgenic B6C3F1 Mice. *Proc. Natl. Acad. Sci. U.S.A.*, 91:6564-6568.
60. Kretz, P.L., Wells, S., and Short, J.M. (1994) Gigapack III: A Single Tube *In Vitro* Lambda Packaging Extract. *Strategies in Molecular Biology* 7:44-45.
61. Knoll, A., Jacobson, D., Kretz, P., Lundberg, K., Short, J., and Sommer, S. (1994) Tissue-Specific Patterns of Spontaneous *LacI* Mutations Recovered From Transgenic Mice. *Mutation Research* 311:57-67.
62. Ashby, J., Short, J.M., Jones, N.J., Lefevre, P.A., Martin, E., Parry, J.M., Burnette, K., Glickman, B.W., and Tinwell, H. (1994) Mutagenicity of *o*-anisidine to the bladder of *lacI*- transgenic B6C3F1 mice: Absence of <sup>14</sup>C or <sup>32</sup>P bladder DNA adduction. *Carcinogenesis* 15:2291-2296.
63. Alting-Mees, M. and Short, J.M. (1994) Rapid Excision Systems. *Strategies in Molecular Biology* 7(3):70-72.
64. Snead, M., Kretz, P.L., Short, J.M. (1994) Methods for Generating Plant Genomic Libraries. *Plant Molecular Biology Manual* H1:1-19.
65. Rogers, B., Provost, G.S., Young, R., Putman, D.L., and Short, J.M. (1995) Intralaboratory Optimization and Standardization of Mutant Screening Conditions Used for a Lambda/*LacI* Transgenic Mouse Mutagenesis Assay (I). *Mutation Research* 327:57-66.
66. Young, B., Rogers, B., Provost, G.S., Short, J.M., and Putman, D. (1995) Interlaboratory Comparison of Liver Spontaneous Mutant Frequency from Lambda/*LacI* Transgenic Mice (Big Blue®) (II). *Mutation Research* 327:67-73.
67. Callahan, J. and Short, J.M. (1995) Transgenic  $\square/lacI$  Mutagenicity Assay: Statistical Determination of Sample Size. *Mutation Research* 327:201-208.
68. Wyborski, D.L., Malkhosyan, S., Moores, J.C., Dyaico, M.J., Perucho, M. and Short, J.M. (1995) Development of a Rat Cell Line Containing a Lambda Shuttle Vector for *In Vitro* Mutagenicity Testing. *Mutation Research* 334:161-165.
69. Snead, M.A., Kretz, P.L., and Short, J.M. (1995) Methods for Generating Plant Genomic Libraries. *Plant Molecular Biology Manual*, Kluwer Academic Publishers: Belgium (H1, 1-19).
70. Robertson, D.E., Mathur, E.J., Swanson, R.V., Marrs, B.L., and Short, J.M. (1996) The Discovery of New Biocatalysts From Microbial Diversity. *SIM News* 46:3-8.
71. Knoll, A., Jacobson, D.P., Nishino, H., Kretz, P.L., Short, J.M., and Sommer, S. (1996) A Selectable System for Mutation Detection in the Big Blue® *lacI* Transgenic Mouse System: What Happens to the Mutational Spectrum Over Time. *Mutation Research* 352:9-22.
72. Wyborski, D.L., DuCoeur, L.C., and Short, J.M. (1996) The Effect of Chromosome Position and Operator Placement on Lac Repressor Control in Eukaryotic Cells and Transgenic Mice. *Environmental and Molecular Mutagenesis*. 28:447-458.
73. Sick, A.J., Fernandez, J. and Short, J.M. (1996) Multiple Purpose Cloning Vectors. *Molecular Biology* (published).

74. Snead, M., Alting-Mees, M.A., and Short, J.M. (1997) cDNA Library Construction for the Lambda ZAP® - Based Vectors. *Methods in Molecular Biology, cDNA Library Protocols*. Humana Press 69:39-51.
75. Snead, M., Alting-Mees, M.A., and Short, J.M. (1997) Clone Excision Methods for the Lambda ZAP® - Based Vectors. *Methods in Molecular Biology, cDNA Library Protocols*. Humana Press 69:53-60.
76. Short, J.M. (1997) Recombinant Approaches for Accessing Biodiversity. *Nature Biotechnology* 15:1322-1323.
77. Nichols, W.S., Geller, S.A., Edmond, V.J., Dyaico, M.J., Sorge, J.A., and Short, J.M. (1998) Hepatocarcinogenesis (Z#2) / mutagenesis during initiation stage. *Mutation Research* 398:143-149.
78. Snead, M., Alting-Mees, M.A., and Short, J.M. (1998) cDNA Library Construction for Lambda ZAP® - Based Vectors. *Methods in Molecular Biology, Plant Virology Protocols*. Humana Press 81:255-267.
79. Bruggeman, T., Short, J.M., and Simms, P. (1998) Diversa: Catalyzing a Revolution. *Industrial Biotech News* 1(1):4,14-15.
80. Deckert, G., Warren, P.V., Gaasterland, T., Young, W.G., Lenox, A.L., Graham, D.E., Overbeek, R., Snead, M., Keller, M., Aujay, M., Huber, R., Feldman, R.A., Short, J.M., Olsen, G.J., and Swanson, R.V. (1998) The Complete Genome of the Hyperthermophilic Bacterium *Aquifex aeolicus*. *Nature* 392:353-358.
81. Sick, A.J., Fernandez, J., Short, J.M., (1998) Multipurpose Cloning Vectors. *Recombinant DNA Principles and Methodologies* 491-522.
82. Li, J., Robertson, D.E., Short, J.M., Wang, P.G. (1999) Chemical and enzymatic synthesis of glycoconjugates. 5: One-pot regioselective synthesis of bioactive galactobiosides using a CLONEZYME thermophilic glycosidase library. *Bioorganic & Medicinal Chemistry Letters* Jan 4; 9 (1):35-8
83. Snead, M.A., Alting-Mees, M.A., Short, J.M. (2000) cDNA Library Construction for the Lambda ZAP® - Based Vectors. *Nucleic Acid Protocols Handbook*, Humana Press Part V:355-365.
84. Sehgal, A.C., Callen W., Mathur E. J., Short, J.M., Kelly, R.M. (2001) Carboxylesterase from *Sulfolobus Solfataricus* P1. *Methods in Enzymology* 330:461-471.
85. Cady, S.G., Bauer, M.W., Callen, W., Snead, M.A., Mathur, E.J., Short, J.M., Kelly, R.M. (2001) Beta-Endoglucanase from *Pyrococcus Furiosus*. *Methods in Enzymology* 330:346-354.
86. Miller, E.S., Kimberley, Parker, N., Liebl, W., Lam, D., Callen, W., Snead, M.A., Mathur, E.J., Short, J.M., Kelly, R.M. (2001) Alpha-D-galactosidases from *Thermotoga* Species. *Methods in Enzymology* 330:246-260.
87. Chhabra, S., Parker, K.N., Lam, D., Callen, W., Snead, M.A., Mathur, E.J., Short, J.M., Kelly, R.M. (2001) Beta-mannanases from *Thermotoga* Species. *Methods in Enzymology* 330:224-238.
88. Parker, K.N., Chhabra, S.R., Lam, D., Callen, W., Duffaud, G.D., Snead, M.A., Short, J.M., Mathur, E.J., Kelly, R.M. (2001) Galactomannanases Man2 and Man5 from *Thermotoga* species: growth physiology on galactomannans, gene sequence analysis, and biochemical properties of recombinant enzymes. *Biotechnology and Bioengineering* Nov 5;75 (3):322-33
89. Gray, K.A., Richardson, T.H., Kretz, K., Short, J.M., Bartnek, F., Knowles, R., Kan, L., Swanson, P.E., Robertson, D.E. (2001) Rapid Evolution of Reversible Denaturation and Elevated Melting Temperature in a Microbial Haloalkane Dehalogenase. *Advanced Synthesis & Catalysis* 2001, 343:607-617.



90. Richardson, T.H., Tan, X., Frey, G., Callen, W., Cabell, M., Lam, D., Macomber, J., Short, J.M., Robertson, D., Miller, C. (2002) A Novel, High Performance Enzyme for Starch Liquefaction: Discovery and Optimization of a Low pH, Thermostable  $\alpha$ -amylase. *Journal of Biological Chemistry* 2002, 277(29), 26501-26507.
91. Zengler, K., Toledo, G., Rappe, M., Elkins, J., Mathur, E.J., Short, J.M., Keller, M. (2002) Cultivating the Uncultured. *Proceedings of the National Academy of Sciences of the United States of America* (2002), 99(24), 15681-15686.
92. Murphy, K.M., Broman, K.W., Ziegler, J.S., Wyborski, D.L., Joe, L.K., Smith, D.W., Thurston, L.M., Stevenson, S.E., McClelland, M., Short, J.M., Mathur, E.J., Varley, J.D. (2002) Successful Breeding Among Free-Ranging 10-Month-Old Gray Wolves (*Canis Lupus*) in Yellowstone National Park, Wyoming. (in press)  
*Yellowstone Center for Resources* (2002). Kerry M. Murphy ([kerry\\_murphy@nps.gov](mailto:kerry_murphy@nps.gov)), P.O. Box 168, Yellowstone National Park, WY 82190.
93. Waters, E., Hohn, M.J., Ahel, I., Graham, D.E., Adams, M.D., Barnstead, M., Beeson, K.Y., Bibbs, L., Bolanos, R., Keller, M., Kretz, K., Lin, X., Mathur, E., Ni, J., Podar, M., Richardson, T., Sutton, G.G., Simon, M., Soll, D., Stetter, K.O., Short, J.M., Noordewier, M. The Genome of *Nanoarchaeum Equitans*: Insights into Early Archaeal Evolution and Derived Parasitism. *Proceedings of the National Academy of Sciences* (2003), Volume 100, No. 22, 12984-12988.
94. Robertson, D.E., Chaplin, A., DeSantis, G., Podar, M., Madden, M., Chi, E., Richardson, T., Milan, A., Miller, M., Weiner, D.P., Wong, K., McQuaid, J., Farwell, B., Preston, L.A., Tan, X., Keller, M., Mathur, E., Kretz, P.L., Burk, M.J., Short, J.M. Exploring Nitrilase Sequence Space for Enantioselective Catalysis. *Applied and Environmental Microbiology* (2004), Volume 70, No. 4, 2429-2436.
95. Palackal, N., Brennan, Y., Callen, W.N., Dupree, P., Frey, G., Goubet, F., Hazlewood, G.P., Healey, S., Kang, Y.E., Kretz, K.A., Lee, E., Tan, X., Tomlinson, G.L., Verruto, J., Wong, V., Mathur, E.J., Short, J.M., Robertson, D.E., Steer, B.A. An Evolutionary Route to Xylanase Process Fitness. *Protein Science* (2004), 13:494-503.
96. Garrett, J.B., Kretz, K.A., O'Donoghue, E., Kerovuo, J., Kim, W., Barton, N.R., Hazlewood, G.P., Short, J.M., Robertson, D.E., Gray, K.A. Enhancing the Thermal Tolerance and Gastric Performance of a Microbial Phytase for Use as a Phosphate-Mobilizing Monogastric Feed Supplement. *Applied and Environmental Microbiology* (2004), Volume 70, No. 5, 3041-3046.
97. YaLi Brennan, Walter N. Callen, Leif Christoffersen, Paul Dupree, Florence Goubet, Shaun Healey, Myrian Hernandez, Martin Keller, Ke Li, Nisha Palackal, Ana Sittenfeld, Giselle Tamayo, Steve Wells, Geoffrey P. Hazlewood, Eric J. Mathur, Jay M. Short, Dan E. Robertson, and Brian A. Steer. *Unusual Microbial Xylanases from Insect Guts*. *Applied and Environmental Microbiology* (2004), Volume 70, No. 6. 3609-3617
98. Kretz, K.A., Richardson, T.H., Gray, K.A., Robertson, D.E., Tan, X., Short, J.M. Gene Site Saturation Mutagenesis (GSSM); A Comprehensive Mutagenesis Technique in Protein Engineering. Robertson, D.E., and Noel, J., eds., *Methods of Enzymology* (2004), Volume 388 (in press).

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM Books

Search PubMed Protein Nucleotide Structure Genome Books  
 CancerChromosomes Conserved Domains 3D Domains Gene Genome Project  
 GENSAT GEO Profiles GEO Datasets HomoloGene Journals MeSH NCBI Web  
 Site NLM Catalog OMIM PMC PopSet PubChem BioAssay PubChem Compound  
 PubChem Substance SNP Taxonomy UniGene Unists for

Limits Preview/Index History Clipboard Details

Brief Summary GI List ASN.1 GenPept GenPept(Full) FASTA XML  
 GPSeq XML TinySeq XML Graph all to file this page to text selected  
 items to clipboard  
 Range: from to Features: SNP CDD MGC HPRD  
 1: BAA22588. Reports endo-1,4-beta-glu...[gi:2467373] BLink, Domains,  
 Links

LOCUS BAA22588 239 aa linear PLN 14-APR-2000  
 DEFINITION endo-1,4-beta-glucanase [Aspergillus oryzae].  
 ACCESSION BAA22588  
 VERSION BAA22588.1 GI:2467373  
 DBSOURCE accession D83731.1  
 KEYWORDS .  
 SOURCE Aspergillus oryzae  
 ORGANISM Aspergillus oryzae  
 Eukaryota; Fungi; Ascomycota; Pezizomycotina; Eurotiomycetes;  
 Eurotiales; Trichocomaceae; mitosporic Trichocomaceae; Aspergillus.  
 REFERENCE 1 (sites)  
 AUTHORS Kitamoto,N., Go,M., Shibayama,T., Kimura,T., Kito,Y., Ohmiya,K. and  
 Tsukagoshi,N.  
 TITLE Molecular cloning, purification and characterization of two  
 endo-1,4-beta-glucanases from Aspergillus oryzae KBN616  
 JOURNAL Appl. Microbiol. Biotechnol. 46 (5-6), 538-544 (1996)  
 MEDLINE 97161783  
 PUBMED 9008887  
 REFERENCE 2 (residues 1 to 239)  
 AUTHORS Kitamoto,N.  
 TITLE Direct Submission  
 JOURNAL Submitted (29-FEB-1996) Noriyuki Kitamoto, Food Research Institute,  
 Aichi Prefectural Government; Shinpukiji-cho 2-1-1, Nishi-ku,  
 Nagoya, Aichi 451, Japan (Tel:052-521-9316, Fax:052-532-5791)  
 FEATURES Location/Qualifiers  
 source 1..239  
 /organism="Aspergillus oryzae"  
 /strain="KBN616"  
 /db\_xref="taxon:5062"  
 Protein 1..239  
 /product="endo-1,4-beta-glucanase"  
 /EC\_number="3.2.1.4"  
 CDS 1..239  
 /gene="celA"  
 /coded\_by="join(D83731.1:501..907,D83731.1:1006..1238,  
 D83731.1:1301..1380)"  
 ORIGIN  
 1 mklslalatl vatafsqelc aqydsasspp ysvnnnlwgq dsgtgsqcvy vdnlsssgaa  
 61 whttwtwngg egsvksysns avtfdkklvs dvqsiptdve wsqdntrvna dvaydlftaa  
 121 dqnhvtysgd yelmiwlary gtiaqigtqi dtatvegthw elwygttiga gaeqktsyfv  
 181 satpintfgg dikkkfddyit skhsfpasaq ylinmqfgte pftggpvtft vpwntasvn  
 //

Disclaimer | Write to the Help Desk  
 NCBI | NLM | NIH

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM Books

Search PubMed Protein Nucleotide Structure Genome Books  
 CancerChromosomes Conserved Domains 3D Domains Gene Genome Project  
 GENSAT GEO Profiles GEO Datasets HomoloGene Journals MeSH NCBI Web  
 Site NLM Catalog OMIM PMC PopSet PubChem BioAssay PubChem Compound  
 PubChem Substance SNP Taxonomy UniGene UNISTS for

Limits Preview/Index History Clipboard Details  
 Brief Summary GI List ASN.1 GenPept GenPept(Full) FASTA XML  
 GPSeq XML TinySeq XML Graph all to file this page to text selected  
 items to clipboard  
 Range: from to Features: SNP CDD MGC HPRD  
 1: CAB06783. Reports endo-1,4-beta-glu...[gi:1870180] BLINK, Domains,  
 Links

LOCUS CAB06783 257 aa linear BCT 22-SEP-1997  
 DEFINITION endo-1,4-beta-glucanase [Thermotoga neapolitana].  
 ACCESSION CAB06783  
 VERSION CAB06783.1 GI:1870180  
 DBSOURCE emb1 locus TNZ86103, accession Z86103.1  
 KEYWORDS .  
 SOURCE Thermotoga neapolitana  
 ORGANISM Thermotoga neapolitana  
 Bacteria; Thermotogae; Thermotogales; Thermotogaceae; Thermotoga.  
 REFERENCE 1  
 AUTHORS Dakhova,O.N., Kurepina,N.E., Zverlov,V.V., Svetlichnyi,V.A. and  
 Velikodvorskaya,G.A.  
 TITLE Cloning and expression in Escherichia coli of Thermotoga  
 neapolitana genes coding for enzymes of carbohydrate substrate  
 degradation  
 JOURNAL Biochem. Biophys. Res. Commun. 194 (3), 1359-1364 (1993)  
 MEDLINE 93356813  
 PUBMED 8352795  
 REMARK (sites)  
 REFERENCE 2 (residues 1 to 257)  
 AUTHORS Zverlov,V.  
 JOURNAL Unpublished  
 REFERENCE 3 (residues 1 to 257)  
 AUTHORS Zverlov,V.  
 TITLE Direct Submission  
 JOURNAL Submitted (27-FEB-1997) Zverlov V., Institute of Molecular  
 Genetics, Russian Academy of Sciences, Kurchatov Sq., Moscow,  
 Russia, 123182

FEATURES Location/Qualifiers  
 source 1..257  
 /organism="Thermotoga neapolitana"  
 /strain="Z2706-MC24"  
 /db\_xref="taxon:2337"  
 Protein 1..257  
 /product="endo-1,4-beta-glucanase"  
 /EC\_number="3.2.1.4"  
 /function="hydrolysis of beta-1,4-glucoside bonds"  
 CDS 1..257  
 /gene="celA"  
 /standard\_name="cellulase"  
 /coded\_by="Z86103.1:3367..4140"  
 /evidence=experimental  
 /transl\_table=11  
 /db\_xref="GOA:P96491"  
 /db\_xref="UniProt/TrEMBL:P96491"

gi1870180embCAB06783.1.txt

ORIGIN

```
1 mveltapgta dfrwndmp1s melnlwnver ytgvtvmrfd gerltfngdv edlsareper
61 yilgypefyy gykpwerhaa egtklplvvs svesftvels feidhmpslp lnfametwlt
121 rekyqveasi gdveimvwfy fneltpggkk vgeytvsfel ngeqkrgiwe lwhaewnwdy
181 lafrlknprv kgrvrfnvkd fldvageyls rstrvkdfdd lyftvweigt efgspetksa
241 rfgwtfnfns idmevkg
```

//

Disclaimer | Write to the Help Desk  
NCBI | NLM | NIH

Jan 7 2005 18:00:44

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM Books

Search PubMed Protein Nucleotide Structure Genome Books  
 CancerChromosomes Conserved Domains 3D Domains Gene Genome Project  
 GENSAT GEO Profiles GEO Datasets HomoloGene Journals MeSH NCBI Web  
 Site NLM Catalog OMIM PMC PopSet PubChem BioAssay PubChem Compound  
 PubChem Substance SNP Taxonomy UniGene Unists for

Limits Preview/Index History Clipboard Details

Brief Summary GI List ASN.1 GenPept GenPept(Full) FASTA XML  
 GPSeq XML TinySeq XML Graph all to file this page to text selected  
 items to clipboard  
 Range: from to Features: SNP CDD MGC HPRD  
 1: CAA93273. Reports endo-1,4-beta-glu...[gi:1297061] BLink, Domains,  
 Links

LOCUS CAA93273 257 aa linear BCT 29-JAN-1997  
 DEFINITION endo-1,4-beta-glucanase [Thermotoga maritima].  
 ACCESSION CAA93273  
 VERSION CAA93273.1 GI:1297061  
 DBSOURCE emb1 locus TMCELAB, accession Z69341.1  
 KEYWORDS  
 SOURCE Thermotoga maritima  
 ORGANISM Thermotoga maritima  
 Bacteria; Thermotogae; Thermotogales; Thermotogaceae; Thermotoga.  
 REFERENCE 1 (residues 1 to 257)  
 AUTHORS Liebl,W., Ruile,P., Bronnenmeier,K., Riedel,K., Lottspeich,F. and  
 Greif,I.  
 TITLE Analysis of a Thermotoga maritima DNA fragment encoding two similar  
 thermostable cellulases, CelA and CelB, and characterization of the  
 recombinant enzymes  
 JOURNAL Microbiology 142, 2532-2542 (1996)  
 REFERENCE 2 (residues 1 to 257)  
 AUTHORS Liebl,W.  
 TITLE Direct Submission  
 JOURNAL Submitted (02-FEB-1996) Liebl W., Technische Universitaet Muenchen,  
 Lehrstuhl f. Mikrobiologie, Arcisstr. 21, 80290 Muenchen, Federal  
 Republic of Germany  
 FEATURES  
 source Location/Qualifiers  
 1..257  
 /organism="Thermotoga maritima"  
 /strain="MSB8 (DSM3109)"  
 /db\_xref="taxon:2336"  
 Protein 1..257  
 /product="endo-1,4-beta-glucanase"  
 /EC\_number="3.2.1.4"  
 /function="hydrolysis of carboxymethyl cellulose,  
 beta-glucan, etc."  
 CDS 1..257  
 /gene="celA"  
 /standard\_name="cellulase"  
 /coded\_by="Z69341.1:1107..1880"  
 /citation=[1]  
 /evidence=experimental  
 /transl\_table=11  
 /db\_xref="GOA:Q60032"  
 /db\_xref="UniProt/TrEMBL:Q60032"  
 ORIGIN  
 1 mvlmtkpqts dfvwnqipls melnlwnike ysgsvamkfd gekitfdadi qnlspkeper  
 61 yvlgypfyfyy gykpwenhta egsklpvpvs smksfsvevs fdihhepslp lnfametwlt  
 121 rekyqteasi gdveimvwfy fnnltpggek iieftipfvl ngesvegtwe lwlaewgdwy

gj1297061embCAA93273.1.txt

181 lafrlkdpvk kgrvkfdvrh fldaagkals ssarvkdfed lyftvweigt efgspetksa  
241 qfgwkfenfs idlevre

//

Disclaimer | write to the Help Desk  
NCBI | NLM | NIH

Jan 7 2005 18:00:44

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM Books

Search PubMed Protein Nucleotide Structure Genome Books  
 CancerChromosomes Conserved Domains 3D Domains Gene Genome Project  
 GENSAT GEO Profiles GEO Datasets HomoloGene Journals MeSH NCBI Web  
 Site NLM Catalog OMIM PMC PopSet PubChem BioAssay PubChem Compound  
 PubChem Substance SNP Taxonomy UniGene UnISTS for

Limits Preview/Index History Clipboard Details  
 Brief Summary GI List ASN.1 GenPept GenPept(Full) FASTA XML  
 GPSeq XML TinySeq XML Graph all to file this page to text selected  
 items to clipboard  
 Range: from to Features: SNP CDD MGC HPRD  
 1: P22669. Reports Endoglucanase I p...[gi:121835] BLINK, Domains, Links

LOCUS P22669 237 aa linear PLN 25-JAN-2005  
 DEFINITION Endoglucanase I precursor (Endo-1,4-beta-glucanase) (Cellulase)  
 (FI-CMCase).  
 ACCESSION P22669  
 VERSION P22669 GI:121835  
 DBSOURCE swissprot: locus GUN\_ASPAC, accession P22669;  
 class: standard.  
 created: Aug 1, 1991.  
 sequence updated: Aug 1, 1991.  
 annotation updated: Jan 25, 2005.  
 xrefs: gi: 217818, gi: 217819, gi: 2287, gi: 2288, gi: 101743  
 xrefs (non-sequence databases): HSSPO74705, InterProIPR008985,  
 InterProIPR002594, PfamPF01670, ProDomPD004316  
 KEYWORDS Cellulose degradation; Direct protein sequencing; Glycosidase;  
 Hydrolase; Pyrrolidone carboxylic acid; Signal.  
 SOURCE Aspergillus aculeatus  
 ORGANISM Aspergillus aculeatus  
 Eukaryota; Fungi; Ascomycota; Pezizomycotina; Eurotiomycetes;  
 Eurotiales; Trichocomaceae; mitosporic Trichocomaceae; Aspergillus.  
 REFERENCE 1 (residues 1 to 237)  
 AUTHORS Ooi,T., Shinmyo,A., Okada,H., Murao,S., Kawaguchi,T. and Arai,M.  
 TITLE Complete nucleotide sequence of a gene coding for Aspergillus  
 aculeatus cellulase (FI-CMCase)  
 JOURNAL Nucleic Acids Res. 18 (19), 5884 (1990)  
 PUBMED 2216782  
 REMARK NUCLEOTIDE SEQUENCE.  
 STRAIN=F-50  
 REFERENCE 2 (residues 1 to 237)  
 AUTHORS Ooi,T., Shinmyo,A., Okada,H., Hara,S., Ikenaka,T., Murao,S. and  
 Arai,M.  
 TITLE Cloning and sequence analysis of a cDNA for cellulase (FI-CMCase)  
 from Aspergillus aculeatus  
 JOURNAL Curr. Genet. 18 (3), 217-222 (1990)  
 PUBMED 2249253  
 REMARK NUCLEOTIDE SEQUENCE, AND PARTIAL PROTEIN SEQUENCE.  
 STRAIN=F-50  
 COMMENT [CATALYTIC ACTIVITY] Endohydrolysis of 1,4-beta-D-glucosidic  
 linkages in cellulose, lichenin and cereal beta-D-glucans.  
 [SUBCELLULAR LOCATION] Secreted.  
 [INDUCTION] By cellulosic materials and hemicelluloses.  
 [MISCELLANEOUS] Will also hydrolyze 1,4-linkages in beta-D-glucans  
 also containing 1,3-linkages.  
 [SIMILARITY] Belongs to the glycosyl hydrolase 12 (cellulase H)  
 family.

gi121835spP22669.txt

-----This Swiss-Prot entry is copyright. It is produced through a collaboration between the Swiss Institute of Bioinformatics and the EMBL outstation -the European Bioinformatics Institute. There are no restrictions on its use as long as its content is in no way modified and this statement is not removed.

-----  
FEATURES                      Location/Qualifiers  
    source                    1..237  
                              /organism="Aspergillus aculeatus"  
                              /db\_xref="taxon:5053"  
    Protein                   1..237  
                              /product="Endoglucanase I precursor"  
                              /EC\_number="3.2.1.4"  
    Region                    1..16  
                              /region\_name="Signal"  
                              /note="Potential."  
                              /evidence=experimental  
    Region                    17..237  
                              /region\_name="Mature chain"  
                              /note="Endoglucanase I."  
                              /evidence=experimental  
    Site                       17  
                              /site\_type="modified"  
                              /note="Pyrrolidone carboxylic acid."  
                              /evidence=experimental

ORIGIN  
    1 mkafhllaa1 agaavaqqaq lcdqyatytg gvytinnnlw gkdagsgsqc ttvnsassag  
    61 tswstkwnws ggensvksya nsgltfnkkl vsqisqiptt arwsydnrtgi radvaydlft  
    121 aadinhvtws gdelmiwla ryggvqpigs qiatatvdgg twelwygang sqktysfvap  
    181 tpitsfqgdv ndffkyltqn hgfpasqyl itlqfgtepf tggpatlsvs nwsasvq

//  
Disclaimer | Write to the Help Desk  
NCBI | NLM | NIH

Jan 7 2005 18:00:44



PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM Books

Search PubMed Protein Nucleotide Structure Genome Books  
 CancerChromosomes Conserved Domains 3D Domains Gene Genome Project  
 GENSAT GEO Profiles GEO Datasets HomoloGene Journals MeSH NCBI Web  
 Site NLM Catalog OMIM PMC PopSet PubChem BioAssay PubChem Compound  
 PubChem Substance SNP Taxonomy UniGene UniSTS for

Limits Preview/Index History Clipboard Details

Brief Summary GI List ASN.1 GenPept GenPept(Full) FASTA XML  
 GPSeq XML TinySeq XML Graph all to file this page to text selected  
 items to clipboard

Range: from to Features: SNP CDD MGC HPRD

1: P16630. Reports Endoglucanase S p...[gi:121830] BLink, Domains, Links

LOCUS P16630 264 aa linear BCT 25-OCT-2004  
 DEFINITION Endoglucanase S precursor (Endo-1,4-beta-glucanase S) (Cellulase S).  
 ACCESSION P16630  
 VERSION P16630 GI:121830  
 DBSOURCE swissprot: locus GUNS\_ERWCA, accession P16630;  
 class: standard.  
 created: Aug 1, 1990.  
 sequence updated: Aug 1, 1990.  
 annotation updated: Oct 25, 2004.  
 xrefs: gi: 148389, gi: 148390, gi: 95575  
 xrefs (non-sequence databases): InterProIPR008985,  
 InterProIPR002594, PfamPF01670, ProDomPD004316  
 KEYWORDS Cellulose degradation; Direct protein sequencing; Glycosidase;  
 Hydrolase; Signal.  
 SOURCE Pectobacterium carotovorum  
 ORGANISM Pectobacterium carotovorum  
 Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;  
 Enterobacteriaceae; Pectobacterium.  
 REFERENCE 1 (residues 1 to 264)  
 AUTHORS Saarilahti,H.T., Henrissat,B. and Palva,E.T.  
 TITLE Cels: a novel endoglucanase identified from Erwinia carotovora  
 subsp. carotovora  
 JOURNAL Gene 90 (1), 9-14 (1990)  
 MEDLINE 90337352  
 PUBMED 2379837  
 REMARK SEQUENCE FROM N.A., AND PARTIAL SEQUENCE.  
 STRAIN=SCC3193

## COMMENT

-----  
 This SWISS-PROT entry is copyright. It is produced through a  
 collaboration between the Swiss Institute of Bioinformatics and  
 the EMBL outstation - the European Bioinformatics Institute.  
 The original entry is available from <http://www.expasy.ch/sprot>  
 and <http://www.ebi.ac.uk/sprot>  
 -----

[CATALYTIC ACTIVITY] Endohydrolysis of 1,4-beta-D-glucosidic  
 linkages in cellulose, lichenin and cereal beta-D-glucans.  
 [SIMILARITY] Belongs to the glycosyl hydrolase 12 (cellulase H)  
 family.

## FEATURES

source

Location/Qualifiers

1..264

/organism="Pectobacterium carotovorum"

/db\_xref="taxon:554"

gene

1..264

/gene="cels"

gi121830spP16630.txt

Protein 1..264  
/gene="celS"  
/product="Endoglucanase S precursor"  
/EC\_number="3.2.1.4"  
Region 1..32  
/gene="celS"  
/region\_name="Signal"  
/evidence=experimental  
Region 33..264  
/gene="celS"  
/region\_name="Mature chain"  
/note="Endoglucanase S."  
/evidence=experimental

ORIGIN

1 mqtvntqphr ifrvllpavf sslllssltv saasssndad klyfgnnkyy lfnnvwgkde  
61 ikgwqqtify nspismgwnw hwpsssthsvk aypslvsgwh wtagytensg lpiqlssnks  
121 itsnvtysik atgtynaayd iwfhthtdkan wdssptdelm iwlnidtnagp agdyietvfl  
181 gdsswnvfkg winadngggw nvfsfvhtsg tnsaslnirh ftdylvqtkq wmsdekyiss  
241 vefgteifgg dgqiditewr vdvk

//

Disclaimer | Write to the Help Desk  
NCBI | NLM | NIH

Jan 7 2005 18:00:44

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM Books

Search PubMed Protein Nucleotide Structure Genome Books  
 CancerChromosomes Conserved Domains 3D Domains Gene Genome Project  
 GENSAT GEO Profiles GEO Datasets HomoloGene Journals MeSH NCBI Web  
 Site NLM Catalog OMIM PMC PopSet PubChem BioAssay PubChem Compound  
 PubChem Substance SNP Taxonomy UniGene Unists for

Limits Preview/Index History Clipboard Details

Brief Summary GI List ASN.1 GenPept GenPept(Full) FASTA XML  
 GPSeq XML TinySeq XML Graph all to file this page to text selected  
 items to clipboard

Range: from to Features: SNP CDD MGC HPRD

1: 2NLRA. Reports Chain A, Streptom...[gi:6573594] BLink, Domains, Links

LOCUS 2NLR\_A 234 aa linear BCT 02-NOV-1998  
 DEFINITION Chain A, Streptomyces Lividans Endoglucanase (Ec: 3.2.1.4) Complex  
 With Modified Glucose Trimer.  
 ACCESSION 2NLR\_A  
 VERSION 2NLR\_A GI:6573594  
 DBSOURCE pdb: molecule 2NLR, chain 65, release Nov 2, 1998;  
 deposition: Nov 2, 1998;  
 class: Hydrolase;  
 source: Mol\_id: 1; Organism\_scientific: Streptomyces Lividans;  
 Strain: 66; Gene: Celb; Expression\_system: Streptomyces Lividans;  
 Expression\_system\_strain: 66; Expression\_system\_plasmid: Piaf9,  
 Piaf18;  
 Exp. method: X-Ray Diffraction.  
 KEYWORDS .  
 SOURCE Streptomyces lividans  
 ORGANISM Streptomyces lividans  
 Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales;  
 Streptomycineae; Streptomycetaceae; Streptomyces.  
 REFERENCE 1 (residues 1 to 234)  
 AUTHORS Wittmann,S., Shareck,F., Kluepfel,D. and Morosoli,R.  
 TITLE Purification and characterization of the CelB endoglucanase from  
 Streptomyces lividans 66 and DNA sequence of the encoding gene  
 JOURNAL Appl. Environ. Microbiol. 60 (5), 1701-1703 (1994)  
 MEDLINE 94288649  
 PUBMED 8017952  
 REFERENCE 2 (residues 1 to 234)  
 AUTHORS Sulzenbacher,G., Shareck,F., Morosoli,R., Dupont,C. and Davies,G.J.  
 TITLE The Streptomyces lividans family 12 endoglucanase: construction of  
 the catalytic cre, expression, and X-ray structure at 1.75 A  
 resolution  
 JOURNAL Biochemistry 36 (51), 16032-16039 (1997)  
 MEDLINE 98101362  
 PUBMED 9440876  
 REFERENCE 3 (residues 1 to 234)  
 AUTHORS Sulzenbacher,G., Mackenzie,L.F., Wilson,K.S., Withers,S.G.,  
 Dupont,C. and Davies,G.J.  
 TITLE The crystal structure of a 2-fluorocellotriosyl complex of the  
 Streptomyces lividans endoglucanase CelB2 at 1.2 A resolution  
 JOURNAL Biochemistry 38 (15), 4826-4833 (1999)  
 MEDLINE 99218092  
 PUBMED 10200171  
 REFERENCE 4 (residues 1 to 234)  
 AUTHORS Sulzenbacher,G., Dupont,C. and Davies,G.J.  
 TITLE Direct Submission  
 JOURNAL Submitted (02-NOV-1998)

COMMENT      Revision History:  
               FEB 7 00 Typographical  
               DEC 8 99 Typographical  
               NOV 12 99 Typographical  
               NOV 10 99 Initial Entry.

FEATURES      Location/Qualifiers

source	1..234
	/organism="Streptomyces lividans"
	/db_xref="taxon:1916"
SecStr	1..5
	/sec_str_type="sheet"
	/note="strand 1"
Bond	bond(5,31)
	/bond_type="disulfide"
SecStr	9..14
	/sec_str_type="sheet"
	/note="strand 2"
SecStr	16..21
	/sec_str_type="sheet"
	/note="strand 3"
SecStr	22..25
	/sec_str_type="sheet"
	/note="strand 4"
SecStr	28..36
	/sec_str_type="sheet"
	/note="strand 5"
SecStr	37..47
	/sec_str_type="sheet"
	/note="strand 6"
SecStr	54..59
	/sec_str_type="sheet"
	/note="strand 7"
SecStr	60..63
	/sec_str_type="sheet"
	/note="strand 8"
Bond	bond(64,69)
	/bond_type="disulfide"
SecStr	67..70
	/sec_str_type="sheet"
	/note="strand 9"
SecStr	76..80
	/sec_str_type="sheet"
	/note="strand 10"
SecStr	83..94
	/sec_str_type="sheet"
	/note="strand 11"
SecStr	97..109
	/sec_str_type="sheet"
	/note="strand 12"
SecStr	118..124
	/sec_str_type="sheet"
	/note="strand 13"
SecStr	125..129
	/sec_str_type="sheet"
	/note="strand 14"
SecStr	135..140
	/sec_str_type="sheet"
	/note="strand 15"
SecStr	141..144
	/sec_str_type="sheet"
	/note="strand 16"
SecStr	145..156
	/sec_str_type="sheet"

```

gi6573594pdb2NLRA.txt
SecStr      /note="strand 17"
            157..165
            /sec_str_type="sheet"
SecStr      /note="strand 18"
            170..176
            /sec_str_type="sheet"
            /note="strand 19"
SecStr      177..186
            /sec_str_type="helix"
            /note="helix 1"
SecStr      192..206
            /sec_str_type="sheet"
            /note="strand 20"
SecStr      207..210
            /sec_str_type="sheet"
            /note="strand 21"
SecStr      211..215
            /sec_str_type="sheet"
            /note="strand 22"
SecStr      216..221
            /sec_str_type="sheet"
            /note="strand 23"
ORIGIN
1 dtticepfgt ttiqgryvvq nnrwgstapq cvtatdtgfr vtqadgsapt ngapksypsv
61 fngchytncs pgtdlpvrld tvsaapssis ygfvdgavyn asydiwldpt artdgvnqte
121 imiwnfrvvp iqpigspvgt asvggrtwev wsgngsndv lsfvapsais gwsfdvmdfv
181 ratvarglae ndwyitsvqa gfepwqngag lavnsfsstv etgtpggtdp gdpq
//
Disclaimer | Write to the Help Desk
NCBI | NLM | NIH
Jan 7 2005 18:00:44

```

#### **Example 14: Product by Function**

**Specification:** The specification exemplifies a protein isolated from liver that catalyzes the reaction of  $A \longrightarrow B$ . The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO: 3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following: substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

#### **Claim:**

A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of  $A \longrightarrow B$ .

#### **Analysis:**

A review of the full content of the specification indicates that a protein having SEQ ID NO: 3 or variants having 95% identity to SEQ ID NO: 3 and having catalytic activity are essential to the operation of the claimed invention. The procedures for making variants of SEQ ID NO: 3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO: 3 which have 95% identity to SEQ ID NO: 3 and retain its activity are conventional in the art.

A review of the claim indicates that variants of SEQ ID NO: 3 include but are not limited to those variants of SEQ ID NO: 3 with substitutions, deletions, insertions and additions; but all variants must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO: 3. Additionally, the claim is drawn to a protein which comprises SEQ ID NO: 3 or a variant thereof that has 95% identity to SEQ ID NO: 3. In other words, the protein claimed may be larger than SEQ ID NO: 3 or its variant with 95% identity to SEQ ID NO: 3. It should be noted that “having” is open language, equivalent to “comprising”.

The claim has two different generic embodiments, the first being a protein which comprises SEQ ID NO: 3 and the second being variants of SEQ ID NO: 3. There is a single species disclosed, that species being SEQ ID NO: 3.

A search of the prior art indicates that SEQ ID NO: 3 is novel and unobvious.

There is actual reduction to practice of the single disclosed species. The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3. The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity. One of skill in the art would conclude that

applicant was in possession of the necessary common attributes possessed by the members of the genus.

**Conclusion:** The disclosure meets the requirements of 35 USC §112 first paragraph as providing adequate written description for the claimed invention.